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A quantitative approach towards a better understanding of the dynamics of *Salmonella* spp. in a pork slaughter-line.

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Running title: *Salmonella* in pig slaughter-line

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ABSTRACT

Pork contributes significantly to the public health disease burden caused by *Salmonella* infections. During the slaughter process pig carcasses can become contaminated with *Salmonella*. Contamination at the slaughter-line is initiated by pigs carrying *Salmonella* on their skin or in their faeces. Another contamination route could be resident flora present on the slaughter equipment. To unravel the contribution of these two potential sources of *Salmonella* a quantitative study was conducted. Process equipment (belly openers and carcass splitters), faeces and carcasses (skin and cutting surfaces) along the slaughter-line were sampled at eleven sampling days spanning a period of 4 months.

Most samples taken directly after killing were positive for *Salmonella.* On 96.6% of the skin samples *Salmonella* was identified, whereas a lower number of animals tested positive in their rectum (62.5%). The prevalence of *Salmonella* clearly declined on the carcasses at the re-work station, either on the cut section or on the skin of the carcass or both (35.9%). Throughout the sampling period of the slaughter-line the total number of *Salmonella* per animal was almost 2log lower at the re-work station in comparison to directly after slaughter.

Seven different serovars were identified during the study with *S.* Derby (41%) and *S.* Typhimurium (29%) as the most prominent types. A recurring *S.* Rissen contamination of one of the carcass splitters indicated the presence of an endemic 'house flora' in slaughterhouse studied. On many instances several serotypes per individual sample were found.

The enumeration of *Salmonella* and the genotyping data gave unique insight in the dynamics of transmission of this pathogen in a slaughter-line. The data of the presented study support the hypothesis that resident flora on slaughter equipment was a relevant source for contamination of pork.

Keywords: *Salmonella*; pigs; PCR; quantitative; resident flora; slaughterhouse.

INTRODUCTION

Salmonellosis is an important cause of food-borne human gastroenteritis in most European countries (EFSA, 2010; Valkenburgh et al., 2007). Farm animals and foods of animal origin form an important source of human *Salmonella* infections. In various European countries a significant number of human cases of salmonellosis (up to 25%) is described to be related to the consumption of pork and pork products (EFSA, 2006; van Pelt et al., 2000; Valdezate et al., 2005).

Carrier pigs are a predominant source of *Salmonella* contamination of pig carcasses during the slaughtering process (Alban and Stärk, 2005; Baptista et al., 2010; Berends et al., 1997; Borch et al., 1996;). Pigs may already have *Salmonella* on their skin before entering a slaughterhouse and, despite stringent hygiene procedures during carcass processing, cross contamination to both *Salmonella* positive and – negative carcasses can occur. The slaughter-line itself can become contaminated by faeces of carrier pigs. In addition, the presence of endemic 'house flora' of *Salmonella* has been described for several slaughterhouses (Baptista et al., 2010; Hald et al., 2003; Visscher et al., 2011; Warriner et al., 2002).

European data on the prevalence of *Salmonella* contaminated carcasses and on serotypes of *Salmonella* on the carcasses is available in various papers. For example, Hald et al. (2003) documented that the prevalence of *Salmonella* contaminated carcasses varied between 0 and 8.5% among 1,623 carcasses examined from five different countries. An EFSA study (26 countries; 5,736 carcass samples) reported a prevalence of *Salmonella* positive carcasses of 0–20% (EFSA, 2008). The most frequently isolated serotype in both studies was *S.* Typhimurium.

The aim of this study was to investigate the dynamics of *Salmonella* in a pig slaughtering process and to assess the origin of carcass contamination. Hereto, the prevalence of *Salmonella* contaminated carcasses was determined. In addition, the concentration of this

pathogen was measured at different sites on the pork meat and slaughtering equipment throughout the slaughtering-line by sampling individual carcasses at exsanguination up to the re-work station. *Salmonella* isolates were serotyped and genotyped.

MATERIALS AND METHODS

Slaughterhouse characteristics

The Dutch slaughterhouse investigated in this study was partly automated with robots for pre-cutting, belly opening, rectum drilling, splitting, leaf lard removal, neck cutting and marking. The capacity of the slaughterhouse is 650 pigs per hour, and 5,000-6,000 animals per day. The waiting time for the pigs at the slaughterhouse was as short as possible (not more than 2 h). Before entering the slaughter-line pigs were electrically stunned, sticked on a table, scalded in a tank, dehaired, flamed, wet polished, flamed and wet polished for a second time.

The belly opener cuts open the belly of a carcass and then cleaves the breastbone into two symmetrical parts. The carcass splitter cuts a carcass into two equal halves with a double knife, without cutting the head.

Sampling strategy

Carcass and equipment samples were collected on eleven days over a period of four months. Different herds were sampled on one sampling day, with a preference of two animals per herd, to account for herd variability. In total, 118 pigs and their carcasses were sampled at two steps of the slaughter process (see Fig. 1 for exact sampling sites)). Directly after exsanguination, skin and rectal samples were taken for the detection, enumeration and typing of *Salmonella*. Immediately after exsanguination 4 cork borer samples were obtained from the shoulder of the animal. A sterile hand held cork borer was used to make four incisions on the shoulder. With a sterile scalpel and forceps slices of 5 cm^2 with a thickness of approximately 5 mm, were cut from the carcass. The four tissue samples, representing a total of 20 cm^2, were collected in one sterile plastic bag, constituting one sample. In addition, a rectal sample was taken from the same animal with a sterile swab (Transwab, Medical Wire and equipment Co.

Ltd., Corsham, Wilts., England), which was immediately placed in 6 ml Buffered Peptone Water (BPW; bioTRADING Benelux B.V., Mijdrecht, The Netherlands).

The carcasses sampled at exsanguination were tracked in the slaughter-line and sampled again after meat inspection at the re-work station. From the cutting site, ham, back before pelvis, sternum and shoulder muscle were sampled with the cork borer. From the lard side, samples were taken with the cork borer from the back, the jowl, the ham and the belly. These interior and exterior samples were collected separately in two sterile plastic bags. In this way a paired set of 2 × 2 different samples were obtained from each animal; two at exsanguination (shoulder (EE), faeces (FS)) and two after final meat inspection at the re-work station (exterior (RE), interior (RI)).

In the slaughter-line the sets of parallel operating belly openers (BO) as well as the carcass splitters (CS) were sampled prior to the start and at the end of the day, immediately after finishing with the slaughtering process. Blades and other easy to reach contact surfaces from the belly openers and the splitting robots were swabbed on both sites using the Meat/Turkey carcass sampling kit (Nasco, Fort Atkinson, WI). In addition, sterile flexistem brushes were used for sampling of parts of the equipment which were less accessible with the carcass sampling kit.

All samples were cooled on site and transported to the laboratory to be analysed on the same day of collection.

Detection of *Salmonella*

Cork borer samples were weighed after arrival in the laboratory and an equal volume of BPW was added. To rectal swabs, equipment swabs and flexistem brushes 6, 20, and 40 ml of BPW, respectively, was added. Cork borer and equipment swab samples were homogenised for 1 min with a Stomacher 400 (Seward, Worthing, United Kingdom). Rectal swabs and

equipment samples taken with a flexistem brush were vortexed for 30 s. A 5 ml aliquot was removed from each sample and stored at 4 °C for enumeration later (see next section). After addition of 90 ml BPW to the cork bore samples, rectal and equipment swabs, all samples were incubated without shaking at 37 °C for 18 to 20 h.

DNA was isolated from a 1 ml aliquot of the enriched culture, using a Chelex-100 suspension (50–100 mesh; Bio-Rad Laboratories B.V., Veenendaal, The Netherlands) according to the manufacturer's instructions. From the final DNA solution, a 5 µl aliquot was directly used as template in the PCR assay described below.

The *Salmonella* real-time assay described by Malorny et al. (2004), except for the internal amplification control, was used to determine the presence of DNA of this pathogen in the various samples. The 50 µl PCR mixture contained 0.4 µM of the primers ttr-4 and ttr-6, 0.25 µM ttr-5 probe (5'-FAM, 3'-BHQ1), 1×Universal Mastermix (Diagenode sa, Liège, Belgium) and a 5 µl aliquot of the sample DNA. Conditions for the real-time PCR were 95 °C for 1 min followed by 45 cycles of 95 °C for 15 s and 65 °C for 30 s. PCR tests were performed on a iQ^{TM}5 Cycler (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands) and data was analysed using the Bio-Rad iQ5 software (Version 2.0).

Samples that were found positive by PCR were considered to be true positives for the assessment of the *Salmonella* prevalence (the cut off value was set at threshold cycle Ct 40 as result of an internal house validation process).

Enumeration of *Salmonella*

The most probable number (MPN; de Man, 1983) method was used to estimate *Salmonella* numbers in the samples identified as positive by PCR. Three subsequent 10-fold serial dilutions were prepared from the stored 5 ml of the original samples. In triplicate 1 ml of each dilution was added to 9 ml of BPW and enriched for 18±2 h at 37 °C. Three separate and

equally spaced drops of incubated BPW (total 100 µl) were pipetted onto the surface of a Modified Semi-solid Rappaport Vassiliadis (MSRV) medium base plate (Merck B.V., Schiphol-Rijk, The Netherlands) supplemented with Novobiocin (20 mg l^{-1}) (Oxoid B.V., Badhoevedorp, The Netherlands) in a triangular configuration. MSRV plates were incubated at 41.5 °C and examined after 24 and 48 h for suspect *Salmonella* growth. A sterile loop (1 µl) was dipped into the edge of any opaque growth and streaked onto SM® ID2 agar plates (BioMérieux SA, Marcy l'Etoile, France) which were incubated at 37 °C for 24 h for the confirmation of *Salmonella*.

To compute the MPN per ml of BPW, it was assumed that all *Salmonella* were detached from the cork borer sample of the carcass surface and brought into the BPW during stomaching. The MPN per ml were converted to MPN per cm^2. Hereto, it was assumed that the bacteria were homogeneously spread over the carcass skins. *Salmonella* numbers per gram of faeces from the rectal swab data were also assessed. The amount of faeces on the swab was not determined during the sampling experiment. Therefore, a small study was performed afterwards in which 50 swabs were weighted before and after insertion into pigs' rectums. The mean amount of faeces that was found on a swab was used to estimate the number of *Salmonella* per gram faeces, using the MPN per swab.

Statistical data analysis

A beta distribution was used to describe uncertainty about the prevalence estimates of *Salmonella* on site or at day level (Vose, 2000). For further analysis of the quantitative *Salmonella* data, the hypothesis that the variation in the Log of all MPN data at one sampling site for Salmonella-positive carcasses can be expressed by a Normal distribution was verified by visually checking its fit to Normality in a quantile-quantile plot. If, by this test, no deviations from normality could be seen, then the *per day* variation in the MPN data from one

sampling site was expressed by a Log-Normal (μ,σ) distribution. The parameters of this distribution were estimated using maximum likelihood estimation, yielding the estimators $\hat{\mu}$ (mean) and $\hat{\sigma}$ (standard error). Samples that were positive by PCR, but in which no *Salmonella* was detected in the dilution series for the MPN assessment, were taken into account and regarded as censored positives. For the censored numbers, the cumulative Log-Normal (μ,σ) distribution function was used to represent the probability of being an observation below detection limit (Gelman et al., 2004). Such concentration distributions could, however, not be assessed for all days. If most, or all, samples were negative in the MPN dilution series on one day, then $\hat{\mu}$ and $\hat{\sigma}$ could not be estimated. For such data sets only the upper limit of the expected concentration $\hat{\mu}$, as provided by the minimal MPN, is given.

Sero- and genotyping of *Salmonella*

Depending on the *Salmonella* concentrations, one to a maximum of five (representative) isolates from each sample were randomly selected. All isolates were stored at –70 °C until use.

The multiplex PCR described by Lim et al. (2003) was used to discriminate between *S.* Typhimurium and non-Typhimurium serotypes in the numerous isolates from the slaughterhouse. The non-Typhimurium isolates were subsequently serotyped by slide and tube agglutination following the Kauffmann–White scheme (Grimont and Weill, 2007).

Multiple-locus variable-number of tandem-repeat analysis (MLVA) was performed on the (monophasic) *S.* Typhimurium isolates as described previously (Torpdahl et al., 2007) to determine whether the isolates were epidemiologically related. Only one (monophasic) *S.* Typhimurium isolate per sample was analysed by MLVA. The MLVA repeats were calculated and named according to the method described by Lindstedt et al. (2004).

Pulsed-field gel electrophoresis (PFGE) was carried out on *S.* Derby and *S.* Rissen isolates with the XbaI restriction enzyme according to the Pulse-Net protocol (Ribot et al., 2006). Gels were analysed using BioNumerics 6.5 software. A dendrogram was produced using the Dice coefficient and the unweighted pair-group method (UPGMA) with a 1.5% tolerance limit and 1.5% optimisation.

RESULTS

Salmonella screening and enumeration

Salmonella was identified on the skin surfaces of 96.6% of all carcasses sampled at exsanguination (Table 1). The estimated mean concentration ($\hat{\mu}$) of *Salmonella* per day in the samples at this site varied between 0.04 and 1.75 log MPN cm^{-2} (Table 2). Of the rectal swabs taken directly after exsanguination 62.5 % were identified positive, whereas the average number of *Salmonella* was 1.88 ± 1.42 log MPN $g.^{-1}$. At the re-work station, 16.2% and 29.9% of the exterior and interior samples, respectively, were tested positive for *Salmonella* (Table 1). In addition, the pathogen counts were lower in comparison to samples taken at exsanguination, with maximum estimated numbers of *Salmonella* of 0.11 and -0.13 log MPN cm^{-2} on the carcass surface (exterior) and cut section (interior), respectively (Table 2). Of all the samples taken in this study, 44.5% (265/596) were identified as *Salmonella* positive. The prevalence of *Salmonella* on the different carcass sampling sites varied between sampling days (Table 1). For the carcass samples collected at the re-work station, an increase in *Salmonella* prevalence was observed from around the second half of the sampling period (08-06-2009 till 16-06-2009), especially for samples collected from the interior part of the carcass. The prevalence declined again towards the end of the experiment. Within one day no clear increase of *Salmonella* positive samples could be demonstrated, i.e. the prevalence of this pathogen in samples taken in the morning were not different from those obtained in the afternoon (Fig. 2).

Before slaughter, no *Salmonella* could be demonstrated on either belly openers, whereas at the end of slaughter 3 out of 40 samples (7.5%) were tested positive. On one sampling day, *Salmonella* was identified on both belly openers (Table 1).

Samples taken from the carcass splitters were more frequently found to harbour *Salmonella*. More specifically, carcass splitter number 2 (CS2) was repeatedly contaminated with this

pathogenic microorganism. In total, during eight out of the eleven sampling days *Salmonella* was identified on this robot after the end of slaughter. Moreover, on two consecutive days *Salmonella* was already found on carcass splitter 2 at the beginning of the slaughtering process (Table 1). In all cases, equipment swabs and flexistem brushes had equal test results.

***Salmonella* serotypes**

In total, 620 *Salmonella* isolates were obtained from all samples taken during this study. Because *S.* Typhimurium was expected to be the most prevalent serovar in pigs (Hald et al, 2003; EFSA, 2008), the multiplex PCR described by Lim et al. (2003) was used to discriminate *S.* Typhimurium isolates from other serovars. The PCR results revealed that 67.5% of all salmonellae isolated at the slaughterhouse were non-Typhimurium isolates. Because of this very large set, it was decided to serotype the main part (64%). When not all isolates from one sample were typed, the result of the subset of typed isolates was assumed to reflect the serotypes of the non-typed ones.

Overall, seven different serotypes were identified, i.e. *S.* 4,5,12:i:– (from here on called monophasic *S.* Typhimurium), *S.* Bredeney, *S.* Brandenburg, *S.* Derby, *S.* Infantis, *S.* Rissen and *S.* Typhimurium (Table 3). Six serotypes were characterised from the animals entering the slaughterhouse, whereas only five different serovars were identified on the carcasses after slaughtering, and only three serotypes were isolated from the slaughterhouse equipment sampled. The most prominent serovars identified at the carcass at exsanguination and their rectal swabs were *S.* Derby (38%), *S.* Typhimurium (36%) and *S.* Brandenburg (18%) (Table 4). The serotypes frequently isolated from the carcasses at the end of the slaughter-line were *S.* Derby (47%) and *S.* Rissen (25%), whereas *S.* Typhimurium was only found in 18% of the cases. The predominant *Salmonella* serotype isolated at the slaughterhouse varied by day of the study.

Although *S.* Typhimurium was prominently present on the carcasses at exsanguination and to a lesser extent at the re-work station, this serovar was not isolated from the carcass splitters. In contrast on the belly openers *S.* Typhimurium was found in two out of three occasions. Carcass splitter 2 (CS2) was frequently contaminated with serovars Derby (56%) and Rissen (44%).

In 15% of all *Salmonella* positive incidences multiple serovars were isolated from individual samples. This was especially true for carcasses at exsanguination (data not shown).

Salmonella genotypes

At least one *S.* Typhimurium or monophasic *S.* Typhimurium isolate from each individual swab or carcass sample (80 animals, 119 isolates in total) positive for these serovars was typed by multiple-locus variable-number of tandem-repeat analysis (MLVA). Nineteen and three different MLVA types could be distinguished among the *S.* Typhimurium and monophasic *S.* Typhimurium isolates analysed, respectively (Table 5).

In 18 cases the same MLVA type was detected in both the rectal swab and exterior sample at exsanguinations, whereas 5 times different MLVA types were encountered in these samples. The 17 *S.* Typhimurium and monophasic *S.* Typhimurium isolates originating from carcasses at the re-work station matched with MLVA types isolated at exsanguination from the same animals, except in three instance (Table 6; Animals 149_1, 657_1 and 657_2).

The two *S.* Typhimurium MLVA types detected on belly opener 2 (BO2) were also found on *Salmonella* samples originating from the incoming animals on those sampling days. In addition, both of these MLVA types were identified in samples taken at the re-work station (Table 5).

A selection of the *S.* Derby and *S.* Rissen isolates (n=96) were genotyped using PGFE. The dendrogram (Fig. 3) shows that the *S.* Rissen isolates belonged to one indistinguishable type,

whereas the PFGE profiles varied among the *S.* Derby isolates analysed, although one particular *S.* Derby genotype clearly dominated the phylogenetic tree. Isolates belonging to this branch originated from various sampling days and all types of samples taken at the slaughterhouse, except the belly opener. In contrast, one branch with a PFGE pattern very similar to the *S.* Rissen profile contained 5 *S.* Derby isolates isolated only from the carcass splitter but at different sampling days.

From several individual carcasses, *S.* Derby was isolated at two or more sampling sites (Table 4). The phylogenetic tree in Figure 3 includes some of these isolates (in bold). *S.* Derby isolates originating from rectal swabs (FS) and skin samples (EE) showed an identical PFGE pattern in 75% of the cases (n=4), whereas, only different PFGE profiles were encountered among the exsanguination (EE) and the re-work station isolates of the same animal of this serovar (n=8).

DISCUSSION

The prevalence of *Salmonella* contaminated carcasses started with 96.6% at exsanguination and was 35.9% after slaughtering at the re-work station. The level of contaminated carcasses in this study was relatively high, compared to other studies (Bouvet et al., 2003; de Busser et al., 2011; Swanenburg et al., 2001a). This high level of *Salmonella* positive samples gave the opportunity to get a clear picture of the contamination routes.

At the re-work station, over 35% of the carcasses tested were *Salmonella* positive. In 10.3% of all tested carcasses, *Salmonella* was detected on both the cut section and on the skin, 19.7% of the tested carcasses were only contaminated at the cut section, and 6.0% contained *Salmonella* only on the skin. So the slaughter process reduces the number of skin contaminated carcasses from 96.6 to 16.2%. Cross contamination via the slaughter process was responsible for at least 30% of all carcasses, i.e. the carcasses were contaminated at the interior side. These results correspond to data reported by others (Berends et al., 1997; Botteldoorn et al., 2003). However, this cross contamination percentage might be an underestimated value since they do not take into account the genotypic diversity of *Salmonella* serovars. In the present study on the one hand the same MLVA type was found at exsanguination and re-work station (Table 6), but on the other hand it was clearly shown that genotypically different subtypes of the same *Salmonella* serotype can be present on one carcass at exsanguination and at the re-work station (see Fig 3 and Table 5).

An excision technique was used as the sampling method for pig skins and carcasses. In many studies (Botteldoorn et al, 2003; EFSA, 2008; Hald et al., 2003; Oosterom et al., 1985; Swanenburg et al., 2001a, 2001b) dry-wet swabbing was the technique of choice. Comparison of both techniques showed that the excision technique was approximately 10-fold more sensitive, but there seemed to be no linear relationship between the two results (Hutchison et al, 2005; Martínez et al., 2010). In case of low concentrations, swabbing a large area is to be

preferred above excision of a small area (Lindblad, 2007), since the excision techniques only samples 5 cm^2 per excision. The concentration data obtained in this study clearly showed that the level of contamination of the sampled carcasses was high enough to use the excision technique.

The average number of *Salmonella* per carcass was almost 2log lower at the end of the slaughter-line. On the skin (12,000 cm^2) a 10 fold lower number was found, i.e. 3.8 to 0.37 *Salmonella* per cm^2. At the cutting area (3,000 cm^2), the average MPN of *Salmonella* was 0.48 per cm^2. As a consequence, the average number of *Salmonella* per carcass decreased from 44,050 (prevalence × concentration × surface; 0.966 × 3.8 × 12,000) at exsanguination to 1,150 per carcass (0.162 × 0.37 × 12,000 + 0.299 × 0.48 × 3,000) at the re-work station. As 37.5% of all salmonellae on carcasses at the re-work station were found on the cutting edges, cross contamination is responsible for more than 35% of all *Salmonella* on pork carcasses based on bacterial counts.

The seven *Salmonella* serovars identified in this study, i.e. *S.* Bredeney, *S.* Brandenburg, *S.* Derby, *S.* Infantis, monophasic *S.* Typhimurium (*Salmonella* 4,5,12:i:–), *S.* Rissen and *S.* Typhimurium were also described by various other authors on pigs at the slaughterhouse stage (Arguello et al., 2011; Bouvet et al., 2003; de Busser et al., 2011; Hald et al., 2003; Swanenburg et al., 2001a).

At the re-work station, five different serovars were detected, whereas at exsanguination six *Salmonella* serotypes were characterised (Table 3 and 4). Two serovars detected at exsanguination, i.e. *S.* Bredeney and *S.* Infantis, were not detected at the re-work station. It might be possible that the contamination level with these serovars was very low and that they disappeared during the slaughter process. In contrast, one serovar, i.e. *S.* Rissen, was not detected at exsanguination but was detected at the re-work station and on one of the carcass splitters. The companies own monitoring program reflected that this slaughterhouse

encountered hygiene problems during and after the study (data not shown). The serological pattern (Table 3 and 4) clearly indicated complicated contamination routes.

The phenomenon of multiple serovars present in individual samples (15%), especially in those taken from carcasses at exsanguination suggested an underestimation of *Salmonella* serotypes in pork, since routinely only one isolate per sample is serotyped.

In order to determine their origin, isolates of the serovars *S.* Rissen, *S.* Derby and (monophasic) *S.* Typhimurium were subtyped. The results differed per serotype. *S.* Rissen was not detected on any of the incoming pigs. Only one PFGE genotype was found on cutting areas of carcasses at the re-work station and on the carcass splitter on various sampling days. This result strongly suggested that resident house flora was a source of carcass contamination. *S.* Derby showed the characteristics of a cross contaminator as none of the strains detected on a single carcass at the re-work station was detected on the same carcass at exsanguination. Comparing MLVA types of (monophasic) *S.* Typhimurium isolates on carcasses at exsanguination and re-work station revealed that (monophasic) *S.* Typhimurium can originate from pigs carrying *Salmonella* into the slaughterhouse. The observation that some carcasses at the re-work station contained MLVA types that were not detected on the same carcass at exsanguination, again showed that cross contamination from one carcass to another can also have occurred.

In this study the carcass splitter was identified as an important source of *S.* Rissen contamination. In previous assessments the carcass splitter has been considered an unimportant attributive source of *Salmonella*, because of the high infection status of the pigs entering the slaughterhouse, especially, if the splitter is equipped with automatic disinfection between each carcass and faecal contamination during evisceration is controlled (Berends et al., 1997; Borch et al., 1996). However, other reports showed that a significant *Salmonella* contamination via the slaughterhouse environment was caused by the carcass splitter

(Sørensen et al., 1999; Swanenburg et al., 2001a, 2001b). In the present study slaughter equipment apparently contributed also to *Salmonella* on pig carcasses. Despite cleaning and disinfection, one of the robots was repeatedly contaminated with *S.* Rissen. Moreover, once this serovar was even present on this carcass splitter prior to the start of slaughter on that day and over the weekend (Fig 3; *S*. Rissen; CS, 13-07-2009).

In the slaughterhouse studied, cross-contamination contributed significantly to the carcass contamination. Resident flora was detected throughout the study on one of the slaughter robots. The serovar identified, *S*. Rissen, contributed significantly to the contamination at the end of the slaughter-line, whereas it was not found on any of the incoming carcasses. In addition, serovars on carcass at the re-work station were many times other types than the ones detected at exsanguination in skin and faeces samples. The data collected, especially the *Salmonella* enumeration results and the sero- as well as genotyping data, gave unique insight in the dynamics of transmission in a slaughter-line.

The sero- and genotyping data will be compared using a variety of statistical tests and implemented in a tracing scheme to predict the source of *Salmonella* on a carcass at the re-work station (Smid et al., 2011).

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Figure Legends

Fig. 1.

Locations of the various cork borer samples, rectal and equipment swabs taken during the slaughtering process in the pig slaughterhouse investigated.

Fig. 2.

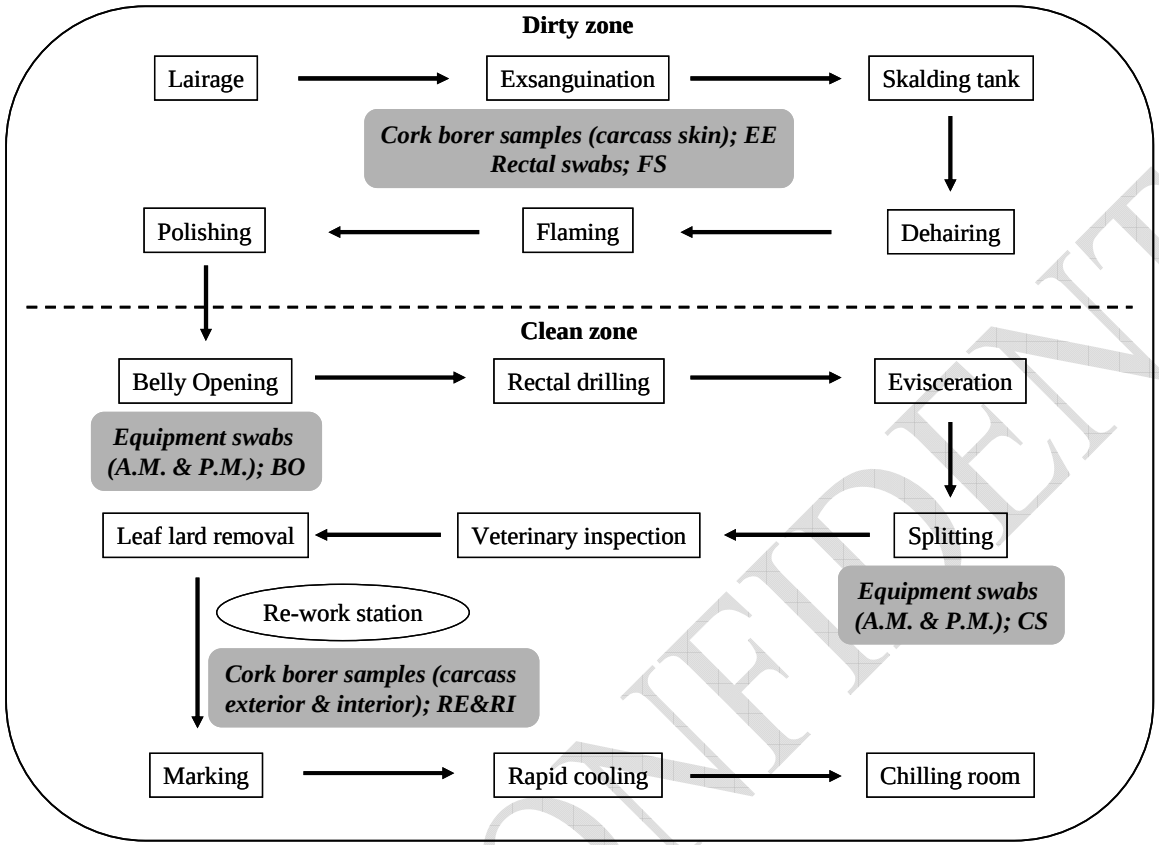
Salmonella prevalence data at the different carcass sampling sites determined by real-time PCR. The black bars represent the samples taken at approximately 11AM; the grey ones indicate the samples taken at approximately 1PM; the white bars show the samples taken at approximately 3PM.

Fig. 3.

PFGE dendrogram of *S.* Rissen and *S.* Derby isolates from the slaughter-line and pigs. BO: Belly opener; CS: Carcass splitter; EE: Exsanguination, exterior; FS: Rectal swab; RE: Re-work station, exterior; RI: Re-work station, interior.

Sample names in bold indicate *S.* Derby isolates from individual carcasses isolated at different stages of the slaughter-line.

515 **Figure 1.**



516 **Figure 2.**

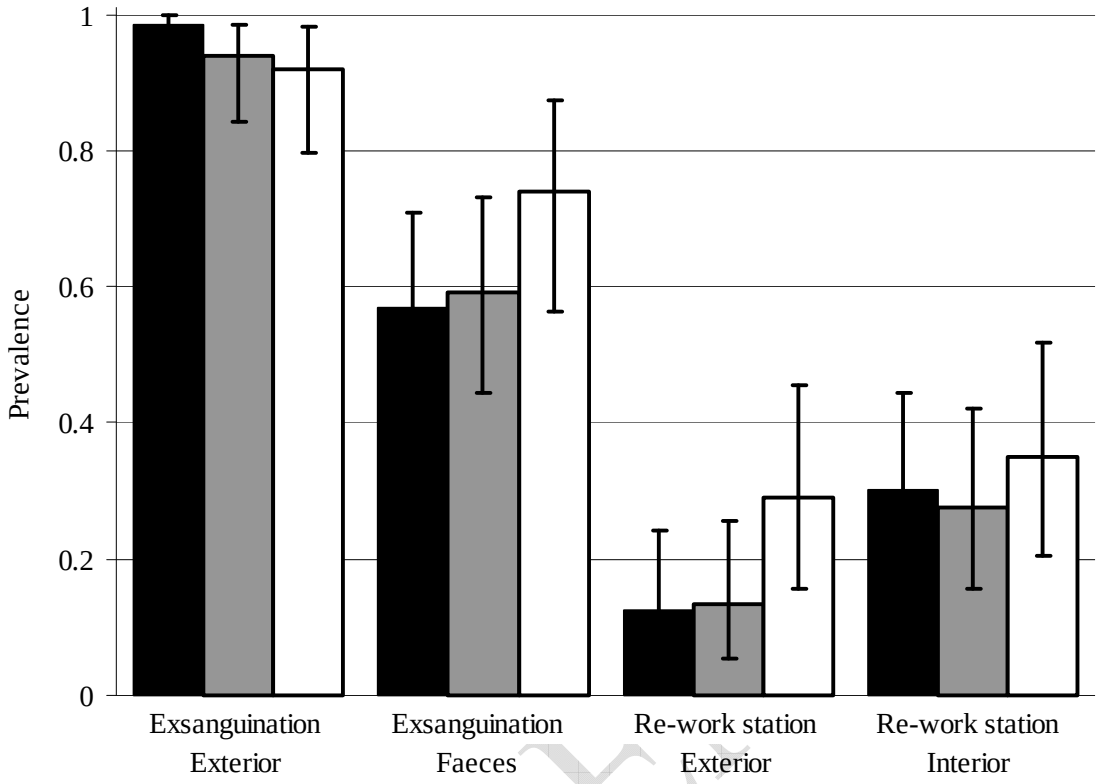
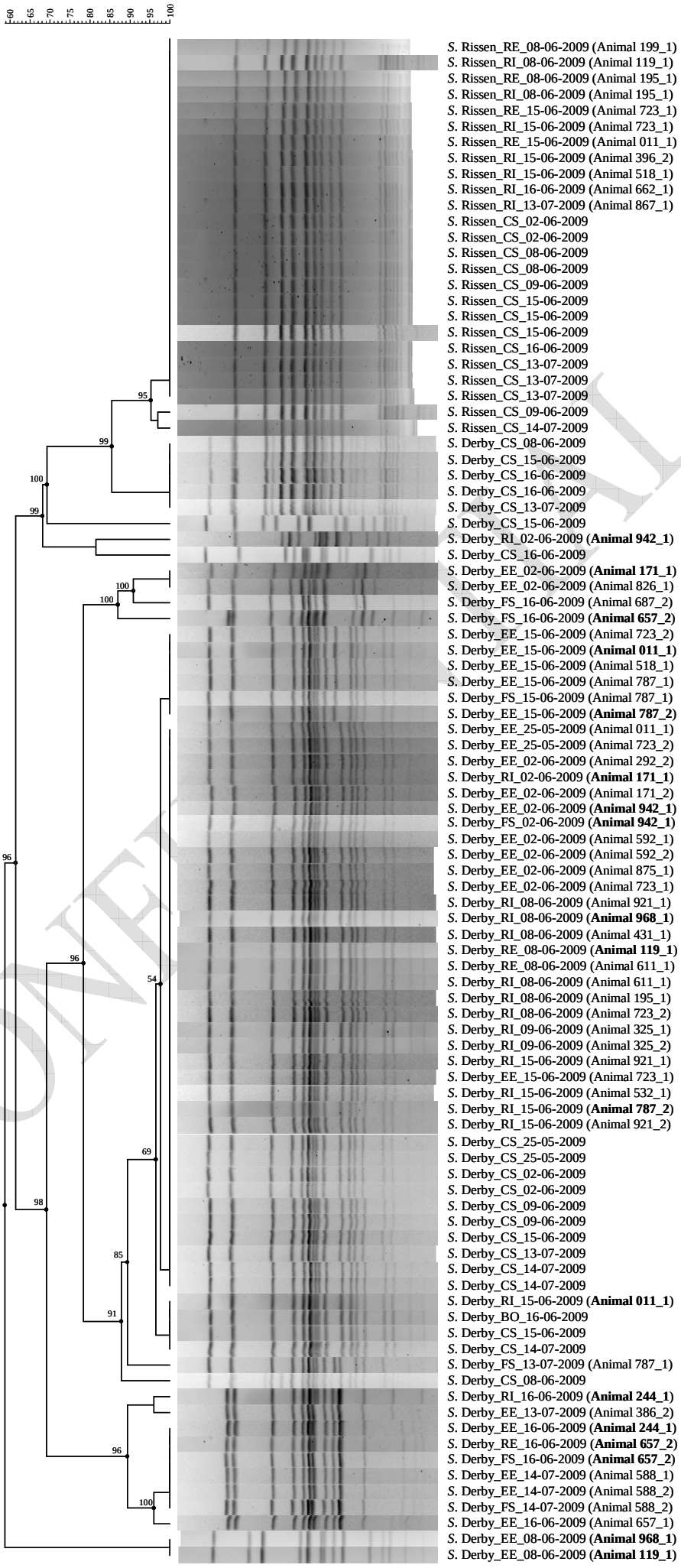


Figure 3.



518 **Table 1**: Number of *Salmonella* positive samples per sampling date and per sampling site determined by real-time PCR.

	Robots – Before slaughter [a]				Exsanguination		Re-work station		Robots – After slaughter [a]			
Date	BO1	BO2	CS1	CS2	Carcass skin	Faeces	Exterior	Interior	BO1	BO2	CS1	CS2
14-04-2009	nd	nd	nd	nd	6/6	nd	2/6	1/6	0/1	0/1	0/1	0/1
20-04-2009	0/1	0/1	0/1	0/1	12/12	10/12	0/12	0/12	0/1	0/1	0/1	0/1
11-05-2009	0/1	0/1	0/1	0/1	8/8	5/8	0/8	0/8	0/2	0/2	0/2	0/2
25-05-2009	0/1	0/1	0/1	0/1	11/12	5/12	2/11	0/11	0/2	0/2	0/2	2/2
02-06-2009	0/1	0/1	0/1	0/1	12/12	6/12	0/12	3/12	0/2	0/2	0/2	2/2
08-06-2009	0/1	0/1	0/1	0/1	12/12	11/12	5/12	8/12	0/2	0/2	0/3	3/3
09-06-2009	0/1	0/1	0/1	0/1	8/8	6/8	2/8	3/8	0/2	1/2	0/2	2/2
15-06-2009	0/1	0/1	0/1	0/1	12/12	8/12	2/12	8/12	0/2	0/2	0/4	4/4
16-06-2009	nd	nd	nd	nd	12/12	8/12	2/12	7/12	1/2	1/2	0/3	3/3
13-07-2009	0/2	0/2	0/2	2/2	12/12	8/12	3/12	2/12	0/2	0/2	0/2	2/2
14-07-2009	0/2	0/2	0/2	2/2	9/12	3/12	1/12	3/12	0/2	0/2	0/2	2/2
Total	***0/11***	***0/11***	***0/11***	***4/11***	***114/118***	***70/112***	***19/117***	***35/117***	***1/20***	***2/20***	***0/24***	***20/24***

519 Note: [a] BO = Belly opener; CS = Carcass splitter; nd = not determined.

520 **Table 2**. Estimated parameters (sample mean, $\hat{\mu}$**,** and standard error, $\hat{\sigma}$) of the Log-Normal

521 probability distribution representing the concentration of positive samples.

	Exsanguination				Re-work station			
	Carcass skin (log MPN/cm^2)		Faeces (log MPN/g)		Exterior (log MPN/cm^2)		Interior (log MPN/cm^2)	
Date	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$
14-04-2009	1.75	0.59	nd		<-0.51		<-0.35	
20-04-2009	0.47	0.49	2.71	0.98				
11-05-2009	0.42	0.71	1.91	1.19				
25-05-2009	0.26	0.75	2.31	1.36	<-0.79			
02-06-2009	0.46	0.84	2.7	0.68			<-0.61	
08-06-2009	0.04	0.91	2.11	1.02	0.11	0.53	-0.13	1.12
09-06-2009	0.52	0.71	2.35	0.78	<-0.52		-0.47	1.04
15-06-2009	0.60	0.61	-3.32	4.96	-0.42	0.82	-0.31	0.72
16-06-2009	0.92	1.33	2.75	1.43	<-0.80		-0.32	0.43
13-07-2009	0.59	1.00	2.61	1.22	-0.98	0.47	-0.37	0.99
14-07-2009	0.34	0.34	2.65	0.6	<-0.83		<-0.59	
Average	***0.58***	***0.75***	***1.88***	***1.42***	***-0.43***	***0.61***	***-0.32***	***0.86***

522

523 Per month:

	Exsanguination				Re-work station			
	Carcass skin (log MPN/cm^2)		Faeces (log MPN/g)		Exterior (log MPN/cm^2)		Interior (log MPN/cm^2)	
Date	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$	$\hat{\mu}$	$\hat{\sigma}$
April-May	0.75	0.80	2.43	1.08	<-0.51		<-0.35	
May-June	0.31	0.83	2.33	1.00	-0.38	0.69	-0.49	1.09
June-July	0.63	0.95	2.25	1.54	-1.30	1.03	-0.39	0.64

524 **Table 3**. *Salmonella* serovars per sampling day determined by multiplex PCR and serotyping.

	Serovar [a]							
Date	BDY	BEG	DRB	INS	mSTM	RSN	STM	Unknown
14-04-2009	0	0	4	6	14	0	3	0
20-04-2009	0	0	55	1	2	0	1	0
11-05-2009	0	0	20	0	6	0	8	0
25-05-2009	0	0	4	0	0	0	16	0
02-06-2009	0	0	35	0	1	5	11	0
08-06-2009	5	16	33	0	0	13	26	1
09-06-2009	0	32	12	0	0	4	14	0
15-06-2009	1	6	41	0	0	20	12	0
16-06-2009	0	0	27	0	0	6	42	1
13-07-2009	3	30	5	0	0	13	24	0
14-07-2009	0	0	19	0	0	2	22	0
% of total	**1.4%**	**13.5%**	**41.0%**	**1.1%**	**3.7%**	**10.1%**	**28.8%**	**0.3%**

525 Note: The multiplex PCR has been described by Lim et al. (2003). The serotyping was
526 performed by slide and tube agglutination following the Kauffmann–White scheme. [a] BDY: *S.*
527 Bredeney; BEG: *S.* Brandenburg; DRB: *S.* Derby; INS: *S.* Infantis;
528 mSTM: monophasic variant *S.* Typhimurium; RSN: *S.* Rissen; STM: *S.* Typhimurium.

529 **Table 4**. *Salmonella* serovars per sampling site determined by multiplex PCR and serotyping.

Sample place[b]		Serotype[a]							
		BDY	BEG	DRB	INS	mSTM	RSN	STM	Unknown
Robots – Before slaughter	BO1	0	0	0	0	0	0	0	0
	BO2	0	0	0	0	0	0	0	0
	SP1	0	0	0	0	0	0	0	0
	SP2	0	0	2	0	0	6	0	0
Exsanguination	Skin	7	63	118	6	17	0	97	1
	Faeces	2	17	47	1	2	0	60	0
Re-work station	Carcass exterior	0	3	5	0	3	10	8	0
	Carcass interior	0	1	37	0	1	12	8	1
Robots – After slaughter	BO1	0	0	1	0	0	0	0	0
	BO2	0	0	0	0	0	0	6	0
	CS1	0	0	0	0	0	0	0	0
	CS2	0	0	45	0	0	35	0	0

530 Note: The multiplex PCR has been described by Lim et al. (2003). The serotyping was
531 performed by slide and tube agglutination following the Kauffmann–White scheme. [a] BDY: *S.*
532 Bredeney; BEG: *S.* Brandenburg; DRB: *S.* Derby; INS: *S.* Infantis; mSTM: monophasic variant *S.*
533 Typhimurium; RSN: *S.* Rissen; STM: *S.* Typhimurium. [b] BO = Belly opener, CS= Carcass splitter.

534 **Table 5**: MLVA types distribution among the various samples.

Serovar	Allele string	Date	Exsanguination		Re-work station		
			Carcass skin	Faeces	Exterior	Interior	BO2
monophasic	02-03-19-14-02	02/06/2009		1			
S. Typhimurium	02-06-04-00-02	14/04/2009	4		3	1	
		20/04/2009	1				
	02-07-06-00-02	11/05/2009	2	1			
S. Typhimurium	02-02-05-00-02	09/06/2009	3		1		1
		13/07/2009			1		
		14/07/2009	7			1	
	02-03-19-01-02	02/06/2009	1				
		13/07/2009	1				
	02-03-19-14-02	25/05/2009	1				
		02/06/2009	1	4			
		08/06/2009	5	5			
		09/06/2009		1			
		15/06/2009	4	2			
		13/07/2009	5	1	1		
	02-05-05-00-02	11/05/2009	5	2			
	02-05-06-00-03	25/05/2009		2			
		08/06/2009		1			
	02-05-20-00-02	16/06/2009	9	3			
	02-06-04-00-02	14/04/2009	1				
	02-07-09-08-03	16/06/2009	2	2	1	1	1
	02-07-10-08-03	16/06/2009		1			
	02-07-11-06-03	02/06/2009	1				
		08/06/2009	1				
	02-08-09-05-03	20/04/2009		1			
	02-11-06-00-03	14/07/2009	1				
	02-17-05-00-02	09/06/2009	1				
	03-02-04-13-02	08/06/2009		2			
	03-03-20-05-02	14/07/2009				1	
	03-04-04-22-02	14/04/2009	2				
		02/06/2009				1	
		16/06/2009				2	
		14/07/2009		1			
	03-08-13-19-02	14/07/2009			1		
	04-01-17-14-02	25/05/2009	8	3	2		
	06-03-00-00-01	13/07/2009		1			

535 Note: [a] BO = Belly opener

536 **Table 6.** All paired occurrences of (monophasic) *S.* Typhimurium typed by MLVA on single

537 carcasses.

Date	Herd_Animal	Origin[a]	Serovar[b]	MLVA allele string
14-04-2009	A_1	EE-RE	mSTM	02-06-04-00-02
	C_1	EE-RE-RI	mSTM	02-06-04-00-02
11-05-2009	396_1	EE-FS	mSTM	02-07-06-00-02
	396_2	EE-FS	STM	02-05-05-00-02
	646_2	EE-FS	STM	02-05-05-00-02
25-05-2009	723_1	EE, FS	STM	04-01-17-14-02, 02-05-06-00-03
	787_1	EE-FS	STM	04-01-17-14-02
	787_3	EE-FS-RE	STM	04-01-17-14-02
	900_1	EE-FS	STM	04-01-17-14-02
	900_2	EE-RE	STM	04-01-17-14-02
02-06-2009	826_1	EE, FS	STM	02-03-19-01-02, 02-03-19-14-02
08-06-2009	431_1	EE-FS	STM	02-03-19-14-02
	611_2	EE-FS	STM	02-03-19-14-02
	921_1	EE, FS	STM	02-03-19-14-02, 03-02-04-13-02
	921_2	EE-FS	STM	02-03-19-14-02
	968_1	EE, FS	STM	02-03-19-14-02, 03-02-04-13-02
15-06-2009	532_1	EE-FS	STM	02-03-19-14-02
	921_1	EE-FS	STM	02-03-19-14-02
16-06-2009	662_1	EE-FS	STM	02-05-20-00-02
	662_2	EE-FS	STM	02-05-20-00-02
	657_1	EE-FS, RI	STM	02-05-20-00-02, 03-04-04-22-02
	657_2	EE, RI	STM	02-05-20-00-02, 03-04-04-22-02
	657_3	EE-FS	STM	02-07-09-08-03
	657_4	EE-FS-RE-RI	STM	02-07-09-08-03
13-07-2009	149_1	FS, RE	STM	03-04-04-22-02, 03-08-13-19-02
	921_1	EE-FS-RE	STM	02-03-19-14-02
	921_2	EE, FS	STM	02-03-19-14-02, 06-03-00-00-01

538 Note: [a] EE: Exsanguination, exterior; FS: Exsanguination, Rectal swab; RE: Re-work station,

539 exterior; RI: Re-work station, interior. [b] mSTM: monophasic *S.* Typhimurium; STM: *S.*

540 Typhimurium.

541